The Growth-Changes in the Mammary Apparatus of Dasyurus and the Relation of the Corpora Lutea thereto.

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With Plates 22 and 23, and 2 Text-figures.

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Introduction.

The following investigation was suggested to me by Professor J. P. Hill, whom I have to thank for the material upon which it was carried out, together with valuable data regarding it, and also for the kindly help he has afforded me throughout the course of the work.

A large amount of work has been done on the growth of the mammary glands during pregnancy in the Eutheria, but, as far as I have been able to discover, no one has hitherto studied the growth of these glands in the marsupials. In this group, however, owing to the comparatively short duration of intra-uterine gestation, one would expect the relation of this growth to the other changes occurring at the same time to be less obscure than in the higher mammals. The animal chiefly investigated was the Australian native cat, Dasyurus viverrinus, a small marsupial which has a breeding season once a year. This is a feature of importance, as it permits the whole of the genital organs, mammary glands and associated structures to return to a condition of complete rest between the breeding periods, whereas in some of the higher animals, for example in the rabbit, a frequent subject of investigation, they are practically in a constant state of activity.

Hitherto only two papers have dealt in any way with the mammary apparatus of Dasyurus. Firstly, Katz (**26**), in 1882, gave a description of the macroscopic appearances in the development of the pouch of the young animal, and secondly, Bresslau (**8**), in 1901, examined and figured one stage of this animal in the course of a general description of the microscopic development of the pouch and teats in the marsupials.

Sandes (**42**), however, has published a valuable account of the development of the corpus luteum in Dasyurus based on material from the collection of Professor J. P. Hill. I have had free access to the same collection, and have been able in a number of cases to study the animals whose ovaries have been described by Sandes. I have also been able to consult Professor J. P. Hill's note-books, and am thus in a position to correlate accurately the stages of development of the embryo, corpus luteum, and mammary apparatus in a way that has not been done previously in any mammal.

Material.

The material consisted of a number of complete pouches of Dasyurus viverrinus, together with the underlying mammary glands, which had been preserved whole in various fixing fluids, that giving the best fixation being picro-corrosive-acetic acid. The pouches were taken from animals in all stages, both before and after parturition, and in each case there was available not only a more or less complete record of the animal for some time previous to killing, but also an exact account of the stage of the embryo, whether in the uterus or in the pouch, so that it was easily possible to ascertain with what stage of embryonal development the growth of the mammary gland corresponded.

In addition to the Dasyurus material, I have examined three stages in the activity of the gland in the domestic cat, one stage in Perameles nasuta, one in the rabbit, and one in the guinea-pig[1] and these have been compared with the corresponding stages in Dasyurus.

The glands were studied by means of serial sections cut through them, and the teat, generally at 8 or 10 μ thick, while for finer histological details sections about half the thickness were employed. The sections were stained by means of Ehrlich's hæmatoxylin and eosin, which stain was found, after experimenting, to produce the best general results. Great care was taken that the changes from absolute alcohol to benzole, and from benzole to paraffin wax, should not be too sudden, so that the glandular tissue should not be injured in any way.

[1] For the last two I have to thank Miss E. M. Simmons, of the London School of Medicine for Women.

The following is a list of the material employed:

Dasyurus Viverrinus.

Description of Material.

Before Ovulation.

Series.

1. . The pouch of a young animal 3 months after birth.
2. . The pouch of a young animal 20 weeks after birth.
3. . Animal getting into heat. Graafian follicles about ripe.
4. . In heat, ova not yet shed.

During Pregnancy.

5. . Ova shed and just entered the uteri.
6. . Ova in one- or two-celled stage.
7. . Ova mostly in two-celled stage, some one-celled.
8. . Embryos in blastocyst stage, 1·24 mm. in diameter.
9. . ,, ,, 1·4 ,, ,,
10. . ,, ,, 3·75 ,, ,,
11. . ,, in vesicle stage 4·5–6·0 mm. in diameter.
12. . ,, ,, 6·5 mm. in diameter; primitive groove stage.
13. . Flat embryos.
14. . Unattached embryos older than 13.

Series		Greatest length.
15.	Embryos just attached to uterus	4·3 mm.
16.	,, some time after attachment	4·7 ,,
17.	,, late uterine stage just before birth	5·75 ,,

After Parturition.

Series		Greatest length.	Head length.
18.	New-born young in pouch just after birth	5·5 mm.	2·5 mm.
19.	Young some hours old	5·75 ,,	3 ,,
20.	Young about 24 hours old	6 ,,	3·25 ,,
21.	,, ,, 36 ,,	6 ,,	3·75 ,,
22.	,, ,, 12 days old	12 ,,	7 ,,
23.	,, ,, 34 ,,	24 ,,	15 ,,
24.	,, 2 months, 5 days after birth	5·7–6 cm.	2·8 cm.
25.	,, 4 months after birth	9·5 ,,	4·5 cm.

After Ovulation not followed by Fertilisation.

26. . Twenty-three unfertilised ova present in the uteri.
27. . Nothing found in uteri. Ovaries with corpora lutea, about 5 days after heat.

Series.
28. . Nothing found in uteri. Ovaries with corpora lutea, about 6 days after heat.
29. . Nothing found in uteri. Ovaries with corpora lutea, about 18 days after heat.
30. . Nothing found in uteri. Ovaries with corpora lutea, about 21 days after heat.

FURTHER MATERIAL.

Series. Description of Material.
31. . Perameles nasuta: New-born young in pouch just after birth.
32. . Domestic cat: Some time after cessation of milk-flow, almost resting.
33. . ,, ,, Young uterine embryos.
34. . ,, ,, After birth, full flow of milk.
35. . Rabbit: Last stages of pregnancy—few days before birth.
36. . Guinea-pig: Towards the end of the milk flow.

The record, from Professor J. P. Hill's note-book, given below, which is that of No. 6 in the foregoing list, may be taken as a typical example of that given with each of the various pouches of D. viverrinus, and a further, more complete record will be given in connection with the changes that occur when ovulation is not followed by pregnancy.

No. 6: Received from Bundaroon 16 . vii . '01: Killed 21 . vii . '01.

16 . vii . '01: Pouch slightly tumid, cloacal margin swollen.

17 . vii . '01: Pouch only very slightly tumid in centre—cloacal margin large and swollen. Male in; Copulation 1–5 p.m.

19 . vii . '01: Pouch very slightly tumid, cloaca still swollen.

20 . vii . '01: Pouch tumid centrally; slightly moist, very dirty.

21 . vii . '01: Pouch tumid; cleaned centrally, but dirty round margin; sebaceous glands not showing. Killed 4 days after copulation.

Left uterus 1·8 × 1·6 × ·7 c.mm. with 11 ova.

Right uterus 1·6 × 1·5 × ·75 c.mm. with 8 ova. one and two celled.

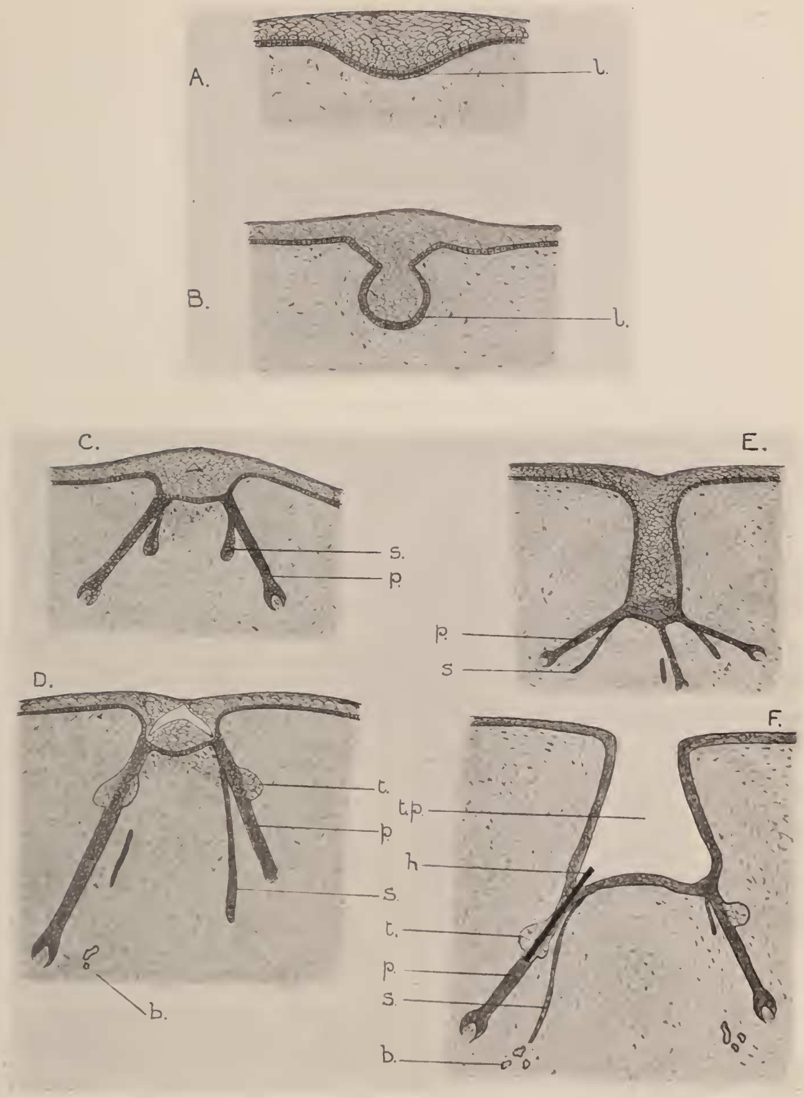
In order to facilitate comparison, all the figures have been drawn at the same magnification by means of a camera lucida with a $\frac{1}{12}$ in. oil-immersion objective and a No. 2 Zeiss ocular. The photo-micrographs also, with the exception of the first, are all taken at a uniform magnification of 16 diameters, and the reproductions are from the untouched negatives. I have to express my thanks to Mr. F. Pittock, of the Zoological Department of this College, for his valuable assistance in the preparation of these photographs. For convenience of description, the material investigated is arranged under six headings: (1) Formation of the gland; (2) the adult gland; (3) changes prior to ovulation; (4) changes during pregnancy; (5) changes after parturition; and (6) changes when ovulation is not succeeded by pregnancy.

Formation of the Gland.

Bresslau (**8**) has shown that the earliest recognisable stage in the development of the mammary organs of the marsupials appears in the form of small lenticular thickenings of the Malpighian layer of the epidermis (see Text-fig. 1, A). Each of these lenticular structures foreshadows one of the teats with its corresponding glands in the adult, and consequently it may be distinguished as the teat anlage. This latter then proliferates, and assumes a club-shaped form (see Text-fig. 1, B), while the cutis around it thickens and forms areolar tissue. The club-shaped condition is maintained for some time, during which the pouch-formation commences.[1] Thus far the development of the teat anlage follows a similar course in all the marsupials, but from this stage onwards Bresslau recognises two different types. In the first, the club-shaped structure alters in form and again becomes more flattened, and while it is still small a crescentic space appears within it (see Text-fig. 1C). The thickened epidermal walls of this space are entirely constituted by the cells of the original

[1] It is not the purpose of the present note to deal with the growth and homologies of the pouch, a subject which has been fully treated in a recent paper by Bresslau (**9**).

TEXT-FIG. 1.



The Formation of the Mammary Glands in Marsupials. A. Lenticular stage; B. club-shaped stage, common to all marsupials. C. and D. Later stages in the development of the first type. E. and F. Later stages in the development of the second type. *b*. Branching hollow part of secondary outgrowth. *h*. Portion of a hair. *l*. Layer of columnar cells. *t.p.* Teat pocket. *p*. Primary outgrowth, i.e. hair-follicle. *s*. Secondary outgrowth, i.e. mammary gland anlage. *t*. Tertiary outgrowth, i.e. sebaceous gland anlage. Figs. A–E are adapted from Bresslau (8). Fig. F is from my own preparations of Dasyurus.

knob-area. In the second the club-shaped anlage grows markedly inwards, and thus increases greatly in length (see Text-fig. 1, E), and finally a hollow appears within it, transforming it into a deep cavity, the teat-pocket (see Text-fig. 1, F). It is to the second type that Dasyurus viverrinus belongs. From this stage onwards we find that all marsupials follow a similar course of development.

The deep aspect of the club-shaped anlage is formed by a layer of columnar cells, which have remained passive during the preceding changes. Now, however, they give rise to solid bud-like outgrowths—the "primary outgrowths"—which grow outwards from the deeper parts of the anlage into the surrounding tissue (see Text-fig. 1, D and F). From these original outgrowths arise similar solid projections—the "secondary outgrowths." The primary outgrowths become transformed into hair-follicles, which produce strong hairs, and which also give rise to their appropriate sebaceous glands, these latter arising from the follicles as tertiary outgrowths. The secondary outgrowths, the anlagen of the mammary glands, grow into the surrounding tissue as solid cellular cords, which, however, become hollow and slightly branched at their distal ends.

In the pouch young of D. viverrinus three months after birth, I find that the marsupium is already laid down as a small, shallow, circular depression, in which are situated the teat anlagen. The anlagen, which in the specimen under consideration (No. 1) were six in number—three on each side of the middle line—have the form of hollow invaginations of the epidermis. This latter is considerably thickened in these teat areas, so that it forms a many-layered stratified epithelium. Each of the teat anlage is provided with six very strong hairs, whose follicles run down from the base of the epidermal invagination into the underlying tissue. These follicles constitute the primary outgrowths of Bresslau (see above). From the upper part of each primary outgrowth there arises a cellular cord, the secondary outgrowth. This is solid proximally, but in its deeper part it becomes hollow, and gives off a few short hollow branches. These latter are the mammary tubules. They are lined by a double-layered epithelium, the inner layer of which consists of somewhat cubical cells (flatter, however, than the cells of the corresponding layer in the adult) and the outer layer of more flattened cells.

At a slightly later stage, in a pouch young twenty weeks after birth, the six strongly built hairs emerging from the teat anlage are still readily distinguishable. In section the hair is seen running into the primary outgrowth, and from the wall of this latter comes off the solid secondary outgrowth, which becomes hollow and branched in its deeper part to form the mammary tubule (fig. 1). The tubules have increased slightly in size and in numbers, and the lumen, at first confined to their distal extremities, has extended a little way along the solid cord towards the primary sprout. The wall of these tubules is in the characteristic two-layered condition, the cells of the inner layer being somewhat more cubical than those of the outer (fig. 11). From the upper part of the hair-follicle, just below the origin of the secondary outgrowth, another bud (the tertiary outgrowth) is originating which will give rise to the sebaceous gland.

Each of the hair-follicles opening into the teat-pocket gives off one secondary outgrowth, and as there are six of these strong hairs, there are also six mammary gland anlagen. In the adult Dasyurus we find that each teat has opening on it six main milk-ducts, and so the hairs in the teat anlagen of the embryo are the same in number as the main milk-ducts, in the fully grown animal.

Further, the distal, hollow part of the secondary outgrowth is lined by a double-layered epithelium, which is also characteristic of the tubules of the adult gland in repose. The solid cord of cells, on the other hand, is many cells in diameter, and it is characteristic of the main ducts that their walls are always more than two cells thick. Thus we may infer that the proximal part of the secondary outgrowths gives rise to the main milk-duct, while the terminal branches form the ramifying mammary tubules of which the adult gland is composed, and from which the secretory alveoli develop.

At a later stage of development than is described above, the hollow of the tubules extends upwards along the solid cord, which is thus transformed into a duct, and comes to open into the upper end of the lumen of the follicle.

From this condition of the gland and teat the transition to the adult form is fairly straightforward, and may be conveniently divided into three stages: (1) The strong hair with its follicle and outgrowing sebaceous gland completely disappears. This, of course, leaves the duct of the mammary gland opening directly into the teat pocket. (2) The mammary tubules increase greatly in length, and become more ramified. (3) The original epidermal invagination, forming the teat pocket, is everted to form the definitive teat.

The Adult Gland.

The resting pouch in the adult is a well-marked, though shallow, almost circular depression, containing as a rule six teats. This number, however, is subject to slight variation, for in the thirty pouches I examined, one (No. 3) had only five teats, with no trace of the sixth, another, (No. 13) had seven, and two (Nos. 6 and 7) had eight. The teats are arranged on either side of a slightly raised ridge occupying the middle line, and are usually situated nearer to the anterior end of the pouch. On the apex of the teat open a number of main milk-ducts (normally six, see fig. 6), which run down some way below the skin before branching into a large number of ramifying mammary tubules. The lining of the main ducts running through the teat, from their external openings up to the point at which they branch, consists of a stratified epithelium four or five cells deep (fig. 12). The branched mammary tubules of the resting gland, on the other hand, are lined by a double layer of cells, similar in appearance to those already described in the pouch young.

The skin lining the pouch is especially characterised by the presence of numerous sebaceous and sweat-glands, which, even in the resting pouch, are larger than those in other parts of the body. They undergo marked hypertrophy during pregnancy, and it is an interesting fact that this hypertrophy runs fairly parallel with that of the mammary apparatus, but slightly in advance of it. Fig. 2 shows a typical section through the teat region of the pouch, with the mammary glands in repose, and gives a good idea of the relation between these and the sebaceous and sweat glands overlying them. The inner side of the whole gland is limited by the musculature of the body wall, and also by the cremaster muscle, while the various groups of tubules, both of the sweat glands and of the lobules of the milk gland, are embedded in a characteristic adipose tissue.

Von Ebner (**49**) describes the lining of the milk-gland tubules as single-layered in man, and Lane-Claypon and Starling (**31**) also describe a simple epithelium in the case of the virgin rabbit. According to Benda (**5**), however, this epithelium is two-layered in the external parts, but becomes single in the deeper parts of the gland, and finally Brouha (**10**) has described in the domestic cat, the rabbit, and the bat, the epithelium lining these tubules as double throughout. From my own observations I am able to confirm this latter statement with regard to the domestic cat. As in the fœtus, so in the adult Dasyurus, all the branching tubules of the mammary gland are lined by a double layer of cells, at any rate in the resting animal, and for some time after ovulation (fig. 13). The true secretory alveoli of the gland with their simple epithelial lining do not make their appearance until the last few days of pregnancy or until some time after ovulation when this has not been followed by fertilisation.

These observations are in harmony with Benda's conclusion that—"The epithelium of the glandular ramifications of the adult mammary gland in repose is composed of a double layer of cells."

The internal layer presents the appearance of a typical glandular epithelium with its granular cells in a state of slight activity. A trace of a secretion somewhat resembling colostrum is always to be found as a coagulum in the lumen of the tubules and ducts (figs. 12 and 13) until it is removed by the more active secretion of colostrum or milk. It would appear, then, that the gland, quite apart from the proper milk-flow, is the seat of slow secretory activity, although this secretion is quite different in microscopic appearance from true milk.

A well-defined lymph-gland is situated close to the outside of the mammary glands and between them and the underlying muscles of the body-wall. When full grown it is about the size of a small pea, and it appears to be correlated in some way with the activity of the mammary gland, for it enlarges at the same time (fig. 5).

Changes Prior to Ovulation.

Professor Hill's observations show that a slight change occurs in the external appearance of the marsupium before ovulation, in addition to the swelling of the cloacal margin characteristic of heat, the pouch being described as becoming tumid and also slightly moist internally. These external alterations are accompanied by corresponding changes in the microscopic appearance of the sebaceous and sweat-glands of the pouch area.

The appearance of tumidity is caused in large measure by the enlargement of the sebaceous glands, which commences just before ovulation and causes the lips of the pouch to swell.

At the same time the sweat-glands become more active and more coiled. The increase in activity is marked by the epithelial cells becoming more granular and slightly larger, and at the same time the lumen of the gland is found to be partly filled by a secretion which, when it is discharged, no doubt causes the inside of the pouch to become moist.

The mammary gland itself exhibits very little change during this period. In the tubules of the deeper part of the gland are to be found a few structures of doubtful significance, which may or may not represent mitotic figures, but the actual formative growth of the gland does not appear to start until after ovulation.

Changes during Pregnancy.

The Pouch.

It would appear from Professor Hill's notes that the changes which occur in the pouch during pregnancy are very well marked, and give some indication of the stage of development reached by the embryo in the uterus. In the resting animal the pouch is a small circular depression about 10 mm. in diameter and 5 mm. in depth, and it is dry inside. In the period proceeding ovulation its lips become slightly tumid and the interior slightly moist, but after ovulation and fertilisation the changes become much more apparent. The pouch slowly enlarges and loses its circular form, becoming somewhat elongated in an antero-posterior direction. Its margins swell and become tumid, and the interior becomes more moist. During the life of the animal the pouch, of course, becomes very dirty, and so needs to be cleaned before receiving the newly-born young. As soon as the secretion of the cutaneous glands appears in some quantity within the pouch, the mother commences to clean it out, at first centrally, and then outwards towards the margin, until at last the whole interior is quite clean. This cleaning process usually appears to take place during the time while the embryos are in the blastocyst stage. After this, the enlargement still goes on, so also does the production of the secretion of the cutaneous glands, which can sometimes be seen as drops of a reddish fluid in the pouch. The pouch itself becomes lax and flaccid, and on its floor the sebaceous glands begin to show beneath the skin. Towards the end of pregnancy these glands are very large and prominent, the interior of the pouch is extremely moist and has assumed a characteristic pinkish hue, and during the last day or so a watery lymph-like fluid is expressible from the teats. At the time the young are born the pouch is extremely lax and moist, and although subject to considerable variations in size, is about 4–5 cm. in diameter by 2–3 cm. deep.

The Cutaneous Glands of the Pouch Area.

The tumidity is mainly due to the very great growth of the sebaceous glands, and these continue to enlarge until they reach their maximum size at just about the time when the embryos are becoming attached to the wall of the uterus. This point was reached in No. 14, where the sebaceous glands are seen to be of very great size and to occupy practically the whole of the space between the mammary glands and the skin (fig. 3). After this time they do not appear to undergo any further enlargement, but remain constant, not only during the remainder of pregnancy, but also for some months afterwards while the young are still using the pouch. This enormous development of the sebaceous glands is an interesting feature, and it appears to be one of the means by which the pouch is enlarged and deepened, and at the same time made more or less soft for the reception of the young.

The sweat-glands again take part in the general growth, but do not undergo such a marked hypertrophy as the sebacous glands. They appear to reach the height of their activity while the ova are segmenting and while the blastocyst is being formed, and as a result of this activity the whole of the inside of the pouch becomes moistened with a somewhat greasy secretion. This activity is maintained throughout the remainder of pregnancy, although the glands appear to become slightly smaller, and so the inside of the marsupium is made moist to receive the young. The presence of this secretion no doubt facilitates the cleaning of the pouch.

The Mammary Glands.

It is in the mammary glands, however, that the most marked changes occur during this period. The growth is manifested by an increase in the ramifications of the tubules, an increase in the size and activity of the individual cells lining them, and the formation of secretory acini.

The tubules become more and more branched and give rise to numerous solid bud-like outgrowths, which appear in section as small masses of cells on the sides of the tubules, and in which mitotic figures are frequently to be found (fig. 15). Later the lumena of the tubules extend into these bud-like projections, the cells of which become arranged round the extension in a double layer. In this condition the outgrowths constitute the first stage of the alveoli. After these primitive alveoli have been formed in considerable numbers their lumena expand, and the double layer of cells lining them becomes reduced to a single layer of somewhat cubical cells. These structures are now the fully formed secretory alveoli (fig. 16). The larger part of this growth takes place prior to the attachment of the embryo to the uterine wall (stage No. 11). The cells of the inner layer increase very rapidly during the formation of the alveolus, and in consequence the outer layer becomes somewhat discontinuous, and its individual cells become elongated and flattened. It was suggested by Kolossow (**30**), that these cells become transformed into typical smooth muscle-fibres at the end of the growth of the gland, in which case we should have muscular cells derived from the ectoderm. I agree with Bronha (loc. cit.) that this does not appear to be the case, although perhaps it may be possible that they have a contractile function, as was suggested by Benda (loc. cit.).

Bizzozero and Vassale (**7**) pointed out that the epithelial proliferations during the growth of the mammary gland in pregnancy resulted from the mitotic divisions of the original cells, and this observation has been confirmed by most investigators since that time. The hypertrophy in Dasyurus occurs in quite a similar manner, and if the frequency of the occurrence of mitotic figures may be taken as an index of the formative growth of the gland, it would appear that the maximum growth activity is reached while the embryos are in the blastocyst stage (i.e. in Nos. 9 and 10). There are signs of mitoses in all the preparations of the gland during pregnancy, save perhaps the last. They are few in the early stages, but during the blastocyst stage they are fairly frequent. After the attachment of the fœtus they become fewer and fewer, ultimately disappearing altogether, and the large increase in size which occurs after this time is more or less of a mechanical nature. It is brought about by the increase in the size of the individual cells and the marked increase of the lumen in both acini and ducts. An examination of the sections of the gland of a domestic cat, in which there were young embryos present in the uteri, shows that a similar mechanical enlargement occurs after the growth of the gland. The actual formation of glandular tissue, as measured by the presence of mitotic figures in its epithelium, was at a standstill, and the gland possessed small, fully formed, single-layered acini.

Winckler (**50**) has described a membrana propria of the glandular alveoli in the mammary gland in the form of a transparent non-cellular membrane. Kolossinikow (**29**), however, considers it to take the form of a network of anastomosing cells, an opinion also held by Heidenhain (**22**). It is described as a simple epithelial covering by Rauber (**38**) and by Jakowski (**25**) as consisting of several layers of anastomosing cells. My own observations confirm those of Sticker (**45**) and Bronha (loc. cit.), who deny its existence as a separate formation, and an examination of the forming, resting, and growing glands shows that in Dasyurus, at any rate, the so-called membrana propria is not an independent structure. It consists simply of conjunctive fibrillar cells from the stroma, which, during the enormous expansion of the alveoli, have been spread out and brought close to their walls.

It has already been stated that towards the end of pregnancy the glands secrete a substance which is expressible from the teat of the living animal as a clear lymph-like fluid. The coagulum of the secretion is present in the acini and ducts of the gland, and is very similar in appearance to colostrum.

Changes after Parturition.

The Cutaneous Glands of the Pouch Area.

Both the sebaceous and the sweat-glands have reached their maximum size early in pregnancy and after parturition they appear to remain constant and in a state of moderate activity, so that the pouch is kept soft and moist for the contained young.

The Mammary Glands.

Little change, if any, is noticeable in the mammary gland itself immediately after parturition; the gland mass has perhaps enlarged a little in bulk owing to the slight increase in the size of the alveoli.[1] No further increase in the actual number of glandular cells appears to take place after the birth of the young, for very careful searching of the sections failed to reveal any sign of mitotic or amitotic cell division. There is a gradual increase in the size of the alveoli up to the time when the young have been in the pouch four months (No. 25), and at this stage, when fully distended by milk, they are of enormous dimensions compared with the size of the newly-formed alveoli.

As in the case of man the secretion of milk does not occur immediately after parturition. Examination of the sections shows that the birth of the young has no immediate effect on the condition of the mammary glands, which is the same

[1] The condition of the mammary glands in Perameles nasuta just after the birth of the young (No. 31) is very similar to that of the glands of Dasyurus at the same time (No. 18).

just after parturition (fig. 5) as it is just before (fig. 4). In the gland of the animal with newly born young (No. 18), and also of the one with young a few hours old (No. 19) no sign of milk is to be found either in the alveoli or in the ducts, although there is a fair amount of a colostrum-like secretion present.

The first appearance of true milk is in No. 20, where the young are twenty-four hours old, and even here it is confined to the aveoli and is not found in the main ducts. It is not until No. 21, that is, thirty-six hours after birth, that we find the gland and main ducts containing milk. The lining of the gland in this animal presents the appearance of a typical glandular epithelium in full activity, and the individual cells have still a more or less cubical form (fig. 17). The original two-layered condition of the tubules and acini has completely disappeared in the deeper parts of the gland and has given way to a single-layered one, and it is only in the central portions near the base of the teat that we find the tubules adjoining the main ducts with two layers of cells. The acini in the central portions of the gland are already beginning to enlarge considerably, and the ducts also have increased in size (fig. 6).

From this time onward the secretion becomes more and more pronounced, and the individual alveoli become increasingly distended, causing the epithelial cells lining them to be stretched and flattened to a remarkable extent. Fig. 8 shows a part of the gland in No. 25, that is, four months after the birth of the young, when the milk-flow is at its height, and it will be seen that the increase in the size of the gland is enormous. Comparison, however, with the three preceding figures shows that this increase is entirely due to the huge distension of the alveoli of the gland by milk and not to an increase in the number of alveoli themselves. Such an enlargement necessitates a great stretching of the secretory epithelium, which under low magnification has the appearance of thin lines marking the outline of the alveoli (fig. 18). Contemporaneous with this great increase in the gland mass there is also an enlargement of the teat, which has grown from about 2–3 mm. up to 8 mm. in number 24 and 1 cm. in number 25.

In No. 23 the gland is in a very interesting condition, for although it is, on the whole, in a more advanced state than in any of the preceding stages, the alveoli are very much smaller, but still in a fully active secretory condition. On the other hand, the main ducts and their proximal branches are enormously distended, and quite full of milk (fig. P9). This is explained by the fact that the milk is periodically forced into the throat of the embryo by the contractions of the cremaster muscle, and that this particular animal was killed while this feeding process was taking place.

The Nature of Milk Secretion.

Two opposing views have been put forward with regard to the nature of milk secretion: it is maintained, on the one hand, that its production is necrobiotic, and, on the other hand, that it is purely a normal secretory process.

The first view was propounded by Virchow (**48**), who held that the activity of the mammary glands was similar to that of the sebaceous glands, and consisted of a physiological fatty degeneration, in the course of which the epithelial cells fell into the lumen and by their disintegration set free the milk-globules. Langer (**32**), however, came to the conclusion that the death of the epithelial cells played no part in milk formation, which was, on the contrary, an entirely secretory occurrence. This point of view was endorsed by Schmidt (**43**), who thought, however, that after some time necrobiosis set in. An intermediate position between these two extremes was adopted by Heidenhain (**22**) in criticism of a theory propounded by Rauber (**38**), that the leucocytes invaded the alveoli and there underwent degeneration to form milk, and the former maintained that the activity was partly necrobiotic and partly secretory. This writer also pointed out that if the activity was purely necrobiotic it would follow that, in order to provide the solid matter necessary for the production of the milk, the cells of the gland would have to be renewed about five times a day. Heidenhain's theory has been maintained in the researches of Frommel (**16**), Steinhaus (**44**), and Dulcert (**12**), who also state, in support of it, that they have found mitoses in all stages of the activity of the gland, by means of which the cells and nuclei that have been destroyed are replaced. Michaëlis (**36**), with slight modifications of no fundamental importance, also supports the view that the nuclei by their degeneration take an active part in milk-formation, but says they are replaced amitotically.

Contemporaneous with these works, however, we have Bizzozero and Vassale (loc. cit.) and Benda (loc. cit.) still upholding the theory of pure secretion, and contending that no necrobiosis of either the epithelial cells or their nuclei occurs. The latter author attributes the torn edges of the cells lining the alveoli and the presence of nuclei therein to faulty technique, and further states that he has seen no stages of mitosis or amitosis. These views have again been borne out by Unger (**47**).

Considering now the most recent writers on this subject, first we have Simon (**33**), in 1902, who adopts in its entirety the theory of Heidenhain, and states that the replacement of the nuclei takes place by amitosis. He divides the secretory changes into three parts: (1) The phase of cellular secretion, (2) the phase of cellular excretion, and (3) the phase of cell reconstruction, and it is in the second of these he describes the cell as becoming decapitated and often, in addition, losing a nucleus. Next, Bronha[1] (loc. cit.) in 1905 also records this decapitation phenomenon, and comes to the conclusion that the normal constituents of milk are produced by two processes: (1) The fatty parts are produced by an ordinary secretory activity of the glandular cells. (2) The albuminous matter results from a necrobiotic activity in which a part of

[1] This work of Bronha contains a very full bibliography and discussion of the subject up to date, which should be consulted in the investigation of this matter.

both the cytoplasm and the nucleoplasm of the cells is set free into the alveoli.

In the same year Arnold (**2**) pointed out that the parts of the cells which lie in the lumen may be artifacts or may be produced by the energetic sucking of the young causing a lesion of the cell walls. Finally, Bertkau (**6**), in 1907, carried out a series of investigations on suitable fixing and embedding methods, and, from very careful comparisons of the results obtained by various processes, came to the following conclusions on this subject: (1) The appearances described as cell decapitation and the expulsion of nucleoplasm are artifacts due to unsuitable hardening or embedding or to post-mortem changes in the material itself. (2) Suitable methods show the epithelium of the alveoli to be essentially similar to that in other glands. (3) The production of milk is a purely secretory occurrence in no way connected with the necrobiosis, either partial or complete, of the epithelial cells. Finally, it is to be remembered, as Lane-Claypon and Starling (loc. cit.) pointed out, there is no evidence to show that the disintegration of the gland-cells would give rise to the specific constituents of milk.

As a result of my investigations on Dasyurus, the domestic cat, the rabbit, and the guinea-pig, the glands of which were fixed immediately after death, I find myself in entire agreement with the conclusions of Bertkau stated above. I find that Zenker's fluid or picro-nitro-acetic acid is not a satisfactory fixative for the mammary gland, and that picro-corrosive-acetic acid or potassium bichromate and acetic acid gives very good results. Particular care was taken that the changes from one fluid to another during staining and embedding should not be too sudden. The sections studied were about 5 μ thick and the results in the various animals very similar. Where the tissues are not well fixed, and in the places where the knife had a bad edge, the lumena of the alveoli contain pieces of torn cells suggesting that decapitation had occurred. Sometimes also nuclei are present in these places, but it is clear from the condition of the surrounding tissue that the section is suffering from faulty preparation. In the tissues that have not this appearance there is no trace of lacerated cell walls or discharged nuclei, but, on the other hand, the outline of the cell and of the nucleus is quite definite and intact, though the shape of the cell becomes more flattened with the mechanical distension of the alveoli. Prolonged searching failed to show any sign of either mitotic or amitotic division of the epithelial cells or their nuclei. The foregoing summaries show clearly that this multiplication of the glandular cells rests upon very doubtful evidence, as some observers describe it as mitotic and others as amitotic.

It would appear, therefore, that milk is produced as the result of a purely vital activity of the epithelial cells of the alveoli of the mammary gland. These cells do not undergo necrobiosis, partially or completely, at any rate during the flow of milk, and they are not replaced by division either with or without mitosis. Lastly, the nuclei and cell-heads appearing in the gland lumen and the lacerated cell walls of the epithelium are to be regarded in the nature of artifacts and not of normal productions.

Changes when Ovulation is not succeeded by Pregnancy.

Professor J. P. Hill has pointed out (**24**) (c. f. also Marshall, p. 576 [**34**]) that in Dasyurus the changes in the mammary apparatus, even when ovulation is not succeeded by pregnancy, are the same as those occurring in pregnant animals, at any rate during the first stage of pregnancy. This is shown clearly in the following record, which is that of No. 29 in a slightly abbreviated form :

Dasyurus viverrinus (No. 3). Killed, 15 . vii . '01.

18 . vi . '01.—Resting pouch, placed with male.

20 . vi . '01.—Cloacal margin getting tumid.

28 . vi . '01.—Cloacal margin tumid, but pouch also tumid and slightly enlarged. Probably pregnant.

30 . vi . '01.—Pouch only slightly tumid, moist slightly but dirty. A whitish glairy secretion from the cloacal aperture

consisting of refractive granules, round and angular (= secretion of anal glands).

1 . vii . '01.—Much the same, but no secretion.

3 . vii . '01.—Pouch slightly tumid round margin, slightly moist, not cleaned. Fresh male in; no action.

5 . vii . '01.—Pouch tumid, more moist centrally, and looks cleaned but dirty round margin.

10 . vii . '01.—Distinctly tumid but slow in altering.

13 . vii . '01.—Very distinctly tumid, somewhat lax; not very moist, sebaceous glands just showing.

15 . vii . '01.—Sebaceous glands now very prominent. Pouch flaccid and deepened, but not greatly. Killed. Both uteri very large and vascular.

Left uterus, 4·1 × 4·1 × 1·1 cm.
Right uterus, 2·7 × 4·1 × 1·1 cm. } Nothing found.

Altogether, then, the mammary glands of five females in which ovulation was not succeeded by pregnancy were investigated.

Series No. 26.—Twenty-three unfertilised ova in the uteri.

Series No. 27.—Ovaries with corpora lutea about 5 days after heat.

Series No. 28.—Ovaries with corpora lutea about 6 days after heat.

Series No. 29.—Ovaries with corpora lutea about 18 days after heat.

Series No. 30.—Ovaries with corpora lutea about 21 days after heat.

The Cutaneous Glands of the Pouch Area.

The tumidity in the pouch of the animals now being considered is caused in great measure by the enlargement of the sebaceous glands. This enlargement is noticeable soon after ovulation (No. 26), and reaches its maximum a few days after this time (No. 28), when the glands are in a condition comparable to that in the pregnant animals with embryos in the late blastocyst stage. The glands remain constant in size in the rest of the animals in this series.

The sweat-glands also undergo hypertrophy, and continue throughout in a state of moderate activity, just as they do in the pregnant animals. This activity results in the production of the secretion which causes the inside of the pouch to become moist.

The Mammary Glands.

Again we find that the mammary gland itself is the seat of the most marked growth during the period after ovulation in these non-pregnant animals. In No. 26, which had twenty-three unfertilised eggs in the uteri, the gland has in addition to the ordinary tubules of the resting gland, a large number of solid bud-like out-growths. These projections give the gland, when viewed in transverse section, a far more compact appearance than it has in the resting condition, and at the end of the growth period they become transformed into secretory acini.

In the next two examples, Nos. 27 and 28, the ovaries contain well-marked corpora lutea, which are fully formed and about full-grown. The mammary gland is still further enlarged, and composed in its deeper parts of a mass of alveoli, the walls in most of which are lined with a double layer of cells, of which the outer layer has become much flattened. But in a number of cases fully formed acini with a single-layered epithelium are present. The tubules near the main ducts are dilated, and both are full of a secretion that is similar in appearance to colostrum, and was, in one case at any rate (No. 28), expressible as lymph from the test of the living animal. The full extent of this enlargement may be realised by a comparison of fig. 9, which is from a section in series No. 27 with fig. 2, from a section of the resting gland. In series No. 28 the gland is in a still more advanced condition, but the tissue itself has not been so well preserved. The stage of development reached by these two would appear to correspond most nearly to that in Nos. 15 and 16, that is to say, in animals with embryos in a late blastocyst stage or just attached to the uterus.

The whole of this growth is brought about by the mitotic divisions of the original epithelium lining the first tubules, and throughout the sections, more especially in those of No. 28, mitotic figures are to be found in the growing portions of the gland (fig. 14).

There remain now two animals, Nos. 29 and 30, which were killed at a much longer time after ovulation. The record of the first of these has already been given. In the second the record is not so complete, but it would appear from the sections of the mammary glands that the growth of these latter had been carried on to a somewhat later stage. The animal was killed nineteen days after the final copulation.

The sebaceous and sweat-glands in No. 29 show a state of development comparable in all respects with that in animals after parturition, and the pouch was tumid, lax and moist. The mammary gland, too, is also in a condition resembling that found in the stages twenty-four hours and thirty-six hours after the birth of the young. The alveoli are fully formed with their walls one cell thick, and their cells are in a state of slight secretory activity. In the alveoli, the tubules, and the ducts leading into the main ducts is to be found a secretion which looks much more like milk than colostrum, and is in all probability the former, although there is no record that it was expressible from the teat of the living animal.

In No. 30 the sebaceous and sweat-glands resemble those of No. 29, but the mammary gland is in a more advanced condition. The general appearance is somewhat in advance of that of the stage thirty-six hours after birth; for the alveoli are more distended, and the ducts more enlarged. Again we have the appearance of a secretion which is almost certainly milk, and the cells of the single-layered epithelium lining the alveoli are in a state of moderate activity. Examination of a section of this gland (fig. 10) shows that it is in a more advanced state than the glands of animals before parturition, and perhaps corresponds most nearly to those in which milk is being secreted (fig. 6). Both the above cases (Nos. 29 and 30), present another point of similarity with the stages after parturition in that no sign of a multiplication of the glandular cells, either mitotically or amitotically, was to be discovered.

The entire series of growth-changes just described is identical with that which occurs in normally pregnant animals. It would therefore appear that although there was no pregnancy, the growth and activity of the mammary glands and neighbouring glands of the pouch area had reached a stage of development indistinguishable from that which occurs in animals a day or two after parturition. There is also present in these cases a secretion which appears to be milk although it is not so plentiful as after pregnancy.

These facts are of great interest in the consideration of the source of the stimulus which brings about the hypertrophy of the mammary glands during pregnancy.

The Stimulus inciting Growth in the Mammary Glands.

The Nature of the Stimulus.

It is well known that there is an intimate connection between the enlargement of the uterus and the growth of the mammary glands during pregnancy. It would appear that this correlation is due to the production of a hormone affecting both parts simultaneously, which is carried by the blood, although the seat of origin of such an internal secretion is still the subject of much discussion. The earlier idea that the growth of these glands was due to a stimulus conveyed by the nervous connections between mammary glands and uterus, although maintained as recently as 1903 by Basch[1] (**3**), appears to be quite ruled out by recent experiments. Eckhard (**13**) cut all the nerves supplying the mammary gland in the goat without interfering with lactation

[1] This work contains a very full bibliography relating to the physiological aspect of the subject.

after parturition. Ribbert (**40**), transplanted one of the mammary glands in the guinea-pig to the region behind the ear, and this transference did not stop the onset of normal hypertrophy and milk secretion. Golz and Ewald (**18**), removed the lower part of the spinal cord of a pregnant bitch, and Routh (**41**) records the case of a pregnant woman whose spine was crushed at the level of the sixth dorsal vertebra, and in both these instances parturition followed by suckling occurred in the normal manner. Finally there is the case of the pygopogous Bohemian twins (**46**), in which the cardæ equinæ of the two spinal cords are probably separate. One of these, Rosa, conceived and gave birth to a boy on April 17th, 1910, and the breasts of the other, Josepha, enlarged simultaneously with those of her sister during the pregnancy of the latter. After the birth of the child, milk was secreted by the glands in Josepha although she had never been pregnant.

From these facts it would appear that the growth of the mammary gland is due to the presence of some specific inciting substance carried by the blood, and not to the nervous connection between ovary and uterus. In Dasyurus, at any rate, this substance may also act as a stimulus to the adjacent sebaceous and sweat-glands.

The Origin of the Stimulus.

Lane-Claypon and Starling (**31**), as the result of an experimental inquiry into the factors causing the growth of the mammary gland of the rabbit during pregnancy, came to the conclusion that it was due to "the action of a specific stimulus produced in the fertilised ovum." Heape (**21**), however, does not agree with this interpretation that the substance comes from the embryo, and brings forward as evidence against it the facts that (1) "the beginning of the development of the gland dates from some point prior to, or during, pro-œstrus, and occurs normally apart from pregnancy"; and (2) "that full functional development of the gland may be experienced by virgins, and therefore without any stimulus derived from a fœtus." The series of growth changes occurring in Dasyurus after ovulation, not succeeded by pregnancy, has already been described in the preceding section, and its practical identity with that of pregnancy has been pointed out. In this case it is perfectly obvious that the enlargement commences quite apart from fertilisation, and is continued when there is no fertilised ovum present to produce an internal secretion.

Lane-Claypon and Starling (loc. cit.) further state that in their opinion the chief source of the hormone in the early stages of pregnancy may perhaps be located in the chorionic villi. This, however, does not apply to Dasyurus, for here we find that the most active growth-period occurs while the embryos are in the blastocyst stage, and that by far the greatest part of the enlargement, due to the actual increase of glandular tissue, has occurred before the embryo becomes attached to the uterus at all. It has been pointed out above that the increase in size after this time is to be explained largely by the mechanical increase in the size of the lumena of both alveoli and ducts. It may be urged as an objection that although there is no placenta present there is still the trophoblastic ectoderm of the embryo, and this might give rise to the hormone. Although this is possible here, it is quite impossible in the cases of milk secretion by virgins when no fœtus is present. Further, it should be remembered that at puberty there is a marked growth in the mammary gland, though no product of conception or organ of attachment is present. Halban (**19**) also looks to the placenta as the point of origin of the hormone during pregnancy, but says that during puberty and menstruation the changes in the mammary glands are dependent on a chemical substance derived from the ovary, and again, that in unusual pathological cases (e.g. abnormal growths in the ovary) the ovary is able to work in a similar manner to the placenta. Thus it does not seem satisfactory to consider the placenta as the point of origin of the stimulus, nor the presence of a fertilised ovum as necessary for its production. We now have left the theory of Heape (loc. cit.) that the source of the stimulus-exciting development in the mammary glands is in the ovary.

The Importance of the Ovary.

There is good experimental evidence to show that in the higher mammals the ovary is of extreme importance to the commencement of the growth of the mammary glands at all times. Marshall and Jolly (**35**) point out that double ovariotomy before puberty begins has the effect of preventing its onset, with the associated growth of the breasts. According to Dixon (**11**) the same operation after puberty produces a cessation of menstruation, sometimes accompanied by atrophy of the breasts and internal genital organs. Halban (**20**) found that if the ovaries were removed from guinea-pigs shortly after birth the uterus and mammary glands remained undeveloped, and Lane-Claypon and Starling (loc. cit.) have shown that, even during pregnancy in the rabbit, if Porro's operation be performed and the ovaries removed during the first fourteen days (i.e. approximately the first half) the development of the mammary gland ceases and retrogression takes place without a secretion of milk. Therefore it seems we should look to the ovary for the seat of this hormone. Knauer (**28**), as a result of his experiments, came to the conclusion that it is only so long as there is an active functional ovary present that it influences the general metabolism of the body. But it is obvious that this secretion is not produced continuously by the ovary, but only at the periods of heat, for it is only at these times that the mammary glands undergo enlargement. An extremely interesting case bearing on this point is reported by Gellhorn (**17**), who describes a woman with seven nipples in the neighbourhood of the mons veneris, and states also that these abnormally-situated mammary glands yielded a flow of milk at each menstrual period. Puberty is only the commencement of these œstral periods, and pregnancy consequent upon one of them. Now œstrus has been shown by Marshall and Jolly (loc. cit.) to correspond with ovulation, which occurs by the bursting of the Graafian follicles, and is followed by the formation of the corpora lutea. It would seem, then, that it is to these structures, formed after each ovulation, that we must look for the seat of the production of the stimulus causing the growth of the mammary glands.

The view that the corpus luteum is a ductless gland was first suggested by Prenant (**37**), and this has been maintained by most of the workers since his time. Indeed, Regaud and Policard (**39**), in the hedgehog, have demonstrated by means of special methods of staining the presence of specific secretory drops in its cells.

The Functions of the Corpus Luteum.

Gustav Born suggested that the function of this ductless gland was to secure the attachment of the embryo to the wall of the uterus, and accordingly Fraenkel and Cohn (**15**) in 1901 and Fraenkel (**14**) in 1903 performed a number of experiments upon rabbits to elucidate this point. The conclusions they arrived at were, that the corpus luteum is a ductless gland whose function is to regulate the nutrition of the uterus, and to prepare its mucous membrane for the maintenance of the fertilised ovum. Marshall and Jolly (loc. cit.), after a series of experiments on bitches and rats, came to much the same conclusion as Fraenkel and Cohn, namely, that "after ovulation, which takes place during œstrus, the corpus luteum is formed, and this organ provides a further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy." Now Fraenkel (loc. cit.) had found that if the corporea lutea alone were destroyed by cautery at any time during the first six days of pregnancy, while the rest of the ovary was left intact, then pregnancy came to an end. Similar experiments on rabbits have recently been made by Kleinhaus and Schenk (**27**), who found that if the corpora lutea were destroyed during the first nine days of pregnancy abortion invariably followed. So that there appears to be no doubt that the corpus luteum is a ductless gland whose secretion is necessary for the attachment of the fœtus in its early stages.

Sandes (loc. cit.) investigated the formation of this body in Dasyurus viverrinus, and he thinks that it is probably a glandular organ whose secretion influences the genital organs and the organism generally, and that it prevents ovulation (a function previously attributed to the secretion of this structure by Beard [**4**], but only temporarily if no pregnancy occurs. He showed that the formation of the corpus luteum was rapid, and that it persisted during the greater part of the time that the animal was lactating, and it only disappeared when the young animals became independent. This work, as was pointed out in the introduction, furnishes the material for an interesting correlation between the growth of the corpus luteum and of the mammary gland. For the purpose of ready comparison I have arranged in five vertical columns, firstly, the various stages in the growth of the corpus luteum and mammary gland, and secondly, the condition of the embryo, of the corpus luteum, and of the mammary gland corresponding with them. The stages of the corpus luteum are taken from Sandes' list which should be referred to for the full details, the stages of the mammary gland from my own list of material given above, and the stages of the development of the embryo from Professor J. P. Hill's note-books. It should be noted, however, that Sandes divides the growth of the corpus luteum into three stages: (1) The early corpus luteum, i. e. the period immediately after the rupture of the Graafian follicle when it is being formed; (2) later corpus luteum, i. e. after the formation is complete, but while it is still actually increasing in size; and (3) a stage in which it remains practically constant.

The same facts may be illustrated in a more striking manner by means of a comparison between the curves of growth in the two cases.

From the above it will be seen that as soon as the corpus luteum has begun to form the growth of the mammary gland

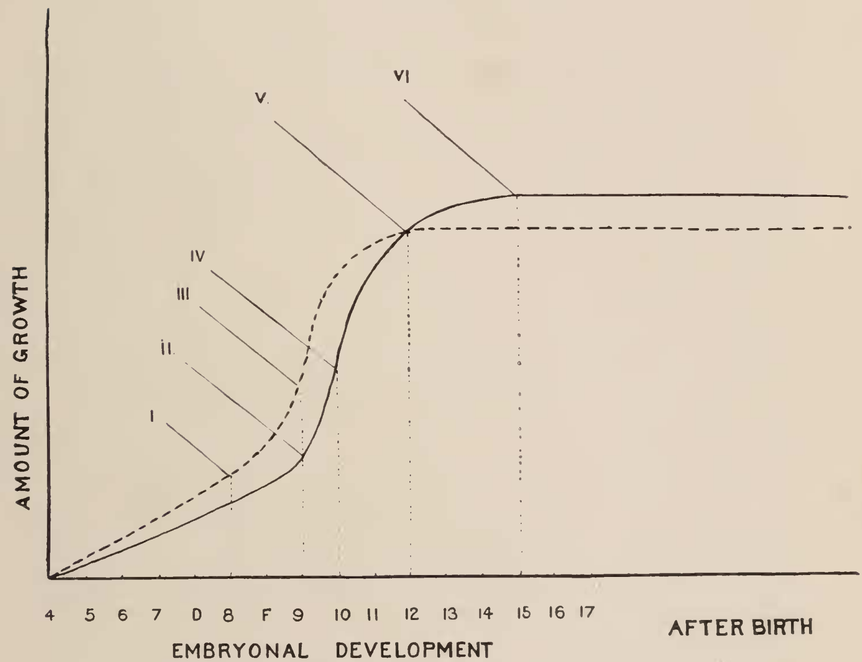
Series of corpus luteum.	Series No. of mammary gland.	Condition of the embryo or ovary.	Condition of corpus luteum.	Condition of mammary gland.
A	3 and 4	Ovary with ripe follicles	None	At rest.
B	5 and 26	Immediately after rupture of Graafian follicles	Commencement of formation	Commencement of growth.
C *	6 and 7 *	Ova in 1- and 2-cell stages	Forming	Growing.
D	—	Ova in 16- and 32-cell stages	Forming	—
E	8	Blastodermic vesicles 1 mm. in diam.	Formed	Growth marked.
F	—	Blastodermic vesicles 2·5–3 mm.	Growing	—
F_1*	9 *	Blastodermic vesicles max. 3·5 mm.	Growing	Increased growth.
G	10	Blastodermic vesicles 3·5–4 mm.	Growing	About height of growth.
H *	12 *	Blastodermic vesicles 6·5–7 mm., primitive streak stage	Large corpora lutea, about the full development of same	Growth slower.
K	17	Late uterine stage, 5·75 mm. embryo	Constant	Constant.
L	18 and 19	New-born young, 3 hours in pouch	Constant	Constant.
M	24 (younger), 25 (older)	Young about 3 months after birth	Constant	Constant.

* In these cases I have definite proof that the animals were identical, and it is very probable that this was also true of the remainder.

commences, and this growth is noticeably increased after the corpus luteum is fully formed—a stage which Sandes has found characterised by the presence of plentiful lutein granules in its cells. The growth in both cases follows a fairly parallel course; that of the mammary glands reaches its maximum in No. 10 and ceases at No. 15, while the height of growth activity in the corpus luteum occurs in No. 9, and it ceases somewhere about No. 12. Then follows a period during which both structures remain practically constant, although there is the mechanical increase in the size of the gland during lactation, and finally the two structures die away together.

In a previous section it has been pointed out that when ovulation is not followed by fertilisation the mammary glands undergo an enlargement similar to that

TEXT-FIG. 2.



The Growth Curves of the Mammary Glands and Corpus luteum in Dasyurus viverrinus. The continuous line represents the growth of the mammary glands. The dotted line represents the growth of the corpus luteum. The numbers along the base line represent stages in the development of the embryo, the details of which have been given in the table above or in the list of material. The letters D and F are intermediate stages in the development of the embryo taken from Sandes' list, and the details of them are given in the above table. (i) End of the formation of the corpus luteum. (ii) Beginning of marked growth in mammary glands. (iii) Height of growth activity in the corpus luteum. (iv) Height of growth activity in the mammary gland. (v) End of growth of the corpus luteum. (vi) End of growth of the mammary gland.

which takes place during pregnancy. It is interesting, therefore, to inquire into the development of the corpus luteum in these cases. Sandes (loc. cit.) in his paper states "that it is probable that the process of corpus luteum formation in Dasyurus ovaries, whose ova are extruded, is the same whether the ovum is fertilised or not." This supposition has proved to be correct [1] and so there is the same marked correlation between the formation and growth of the corpus luteum and the hypertrophy of the mammary glands in non-pregnant as in pregnant animals. It is to be borne in mind also that the stimulus causing growth is not nervous but due to the presence of a hormone, and that in the non-pregnant animals this secretion could not come from either fœtus or placenta.

These considerations make it extremely probable that, at any rate in Dasyurus, we may regard the corpus luteum as a ductless gland producing an internal secretion, which in addition to its other functions is intimately connected with, if not, indeed, the actual inciting cause of, the growth of the mammary gland.

There is considerable evidence to show that a similar statement is true also of the rabbit, for Lane-Claypon and Starling (loc. cit.), in the course of their investigations noted, that the growth of the mammary gland is marked almost immediately after impregnation, and they arrived at the three following experimental results :

[1] In order to substantiate this observation, I have cut serial sections of the ovaries of several animals in which ovulation was not succeeded by pregnancy, including those of Nos. 28, 29, and 30 in the foregoing list of mammary gland material. These were compared with the original slides made by Dr. Sandes and now in the possession of Professor J. P. Hill, and from an examination and comparison of the two series it appears that the process of formation and growth of the corpora lutea is the same in both cases. Dr. Sandes was also good enough to look through the slides for me, and he confirmed his previous opinion that there is no difference between the formation of the corpora lutea in a pregnant and non-pregnant Dasyurus, i. e. between the corpus luteum verum and the corpus luteus spurium.

(1) That if the ovaries and internal genital organs be removed before the fourteenth day, i.e. about the first half of pregnancy, the mammary glands return to a state of rest without giving any milk.

(2) That if the same operation be performed after this time within two days milk is expressible from the nipple, that is to say, that by this time the mammary gland has reached a stage of development at which it is able to become functional.

(3) That "the growth of the ovaries is, however, chiefly marked in the first third or half of pregnancy. After this time the Corpora lutea begin to diminish in size and with them the whole ovaries."

Now the researches of Fraenkel and Cohn (loc. cit.) and those of Kleinhans and Schenk (loc. cit.), have shown that the important factor in the changes during early pregnancy is the corpus luteum and not the whole ovary.

It would therefore appear from a consideration of these facts that in the rabbit the secretion of the corpus luteum is not only necessary for the first stages in the attachment of the embryo, but also is the stimulus causing the growth of the mammary glands, at least up to a point where the gland is sufficiently developed to produce milk.

Since arriving at the foregoing conclusion, my attention has been drawn to a demonstration of Ancel and Bouin (**1**), who have shown that in the rabbit, when the appearance of the corpus luteum followed on coition with a male, part of whose vasa deferentia had been double ligatured for some months, or on the artificial rupture of the follicle, there was a large growth of the mammary glands. This growth was very noticeable by the fourth day, and continued up to the fourteenth day, after which regression set in. It is to be noted that Lane-Claypon and Starling found that removal of the internal genital organs and ovary only produced a regression of the mammary glands without milk secretion if performed before the fourteenth day.

From the evidence adduced above in regard to the rabbit it would seem that the growth of the glands during the last half of pregnancy cannot be due to the corpora lutea, as these bodies are diminishing or have disappeared. It is very probable, however, that the growth after the first fourteen days is due largely to an increase in the size of the individual cells and a distension of the alveoli and ducts, for in the specimen I have examined, No. 35, the formation of new glandular cells has ceased. Now Lane-Claypon and Starling have shown that subcutaneous injections of fœtus and placenta extracts continued for some time produce a marked hypertrophy of the mammary glands. So that there are grounds for thinking that in this animal, at any rate, the presence of a fœtus may have something to do with the continued enlargement of the glands. But it is perhaps a significant fact that in these experiments there is no unequivocal case in which a secretion of milk resulted, although the injections in one case were continued forfive weeks. Further, the whole experiments were somewhat vitiated because there was no allowance made for the growth of the gland occurring at each œstral period.[1]

In considering the question of the seat of origin of the internal secretion inciting growth in the mammary glands, we have to take into account the following facts :

(1) There is a marked correlation between the formation and growth of the corpora lutea and the growth of the mammary glands both in the pregnant and non-pregnant Dasyurus viverrinus.

(2) The experiments of Lane-Claypon and Starling, given above, show that the removal of the ovary while it still has well-developed corpora lutea leads to a regression of the mammary glands without functional activity.

(3) The experiments of Fraenkel and Cohn and of Kleinhaus and Shenk (both referred to previously) have shown the extreme importance of the secretion of the corpora lutea, and not the whole ovary, to the uterine changes in the first stages of pregnancy.

(4) Ancel and Bouin (loc. cit.) showed that a growth of

[1] See Addendum.

the glands followed on the production of corpora lutea in non-pregnant rabbits. This growth went on for fourteen days and then regression set in.

(5) Fourteen days is about the time taken in the formation and growth of the corpus luteum, and after this time it begins to disappear (Lane-Claypon and Starling).

From this evidence it seems we are justified in drawing the following conclusion: The hormone causing the growth of the mammary glands during pregnancy, and at other times, is produced in the corpora lutea.

The Cause of the Secretion of Milk.

It is not intended here to discuss in detail the cause of the secretion of milk in the glands, a question by no means satisfactorily settled, and one that awaits further experimental inquiry, but at the same time a brief reference to it appears to be necessary.

Hildebrandt (**23**) put forward the theory that the fertilised ovum during its development acted as a stimulus to the growth of the mammary glands, and at the same time protected the cells of the latter from autolytic disintegration. On the removal of this check therefore the cells underwent autolysis, and so produced milk. Reasons have been given above, under the section dealing with the changes after parturition, to show that this secretion is not to be regarded as the result of any necrobiotic changes, but is, on the other hand, a purely vital activity. The second part of the theory, i.e. that lactation follows upon the removal of an inhibitory stimulus, has been adopted by Halban (**19**), and also Lane-Claypon and Starling (loc. cit.). The former gives an account of cases of extra-uterine fœtation in which the premature death of the embryo was not followed by its expulsion, and points out that there was a flow of milk from the breast a few days after the death may be supposed to have taken place. He records a similar phenomenon after the death of the fœtus when this occurs in the uterus, and he ascribes the production of milk in both cases to the removal, by death, of the placenta. The latter authors, from a consideration of the results obtained by the removal of the internal genital organs and ovaries (cited above), come to the conclusion that ". . . lactation is due to the removal of the stimulus, which, during pregnancy, occasions the hypertrophy of the mammary glands." Both Halban and Lane-Claypon and Starling regard such a stimulus as arising either in the placenta or the fœtus. There are four classes of facts of which neither of the above theories is a sufficient explanation. Firstly, they do not account for the production of milk in the case of virgins nor in the interesting case reported by Gelhorn (loc. cit.), where milk was produced at each menstruation. Secondly, they do not account for the fact that in the rabbit true milk is obtainable from the gland during the last two days of pregnancy, i. e. before the removal of either fœtus or placenta. Thirdly, the secretion of milk in the newly born young, a subject discussed at some length by Bronha (loc. cit.) tells against the theory that the inhibitory secretion has its origin in the fœtus, as in this case there is no removal of the stimulus and yet there is milk produced. Lastly, they do not account for the large growth of the mammary gland, identical with that taking place in pregnancy, and probably followed by a small secretion of milk, which occurs normally in Dasyurus when ovulation is not followed by fertilisation.

Up to the present, then, there does not appear to be a satisfactory explanation of the causation of milk secretion, and the view that it is due to the removal of an inhibitory stimulus whose origin is the placenta or fœtus, consequent upon parturition, must be regarded as by no means fully established.

General Summary.

Formation of the Gland.—(1) The teat anlagen arise from a proliferation of the cells of the Malpighian layer of the epidermis.

(2) The mammary gland anlage arises as a solid cellular outgrowth from the follicles of especially strong hairs on the primitive teat anlage.

(3) The hairs on the teat anlage are equal in number to the main milk-ducts in the adult teat.

(4) The proximal solid part of the mammary gland outgrowth gives rise to the main milk-duct and the distal, hollow branches to the mammary tubules of the adult.

The Adult Gland.—(1) The fully grown gland in repose consists of six main ducts, with a lining epithelium several cells deep, opening on the teat. The ducts lead down into a large number of ramified twisted tubules whose walls are two cells thick.

(2) It is the seat of continuous, but very slow, secretory activity.

Changes prior to Ovulation.—There is an increase in the size and activity of both the sebaceous and sweat-glands of the pouch area, but very little, if any, growth in the mammary gland.

Changes during Pregnancy.—(1) There is a large increase of mammary gland tissue at this period, resting upon the mitotic division of its epithelial cells, and resulting in the formation of a great number of true alveoli with single-layered walls and a secretion of lymph.

(2) The sebaceous and sweat-glands of the pouch area also hypertrophy and become active, the secretion of the latter appearing as drops of a reddish fluid.

(3) The greater part (practically the whole) of the growth in actual gland-tissue occurs before the attachment of the embryo.

Changes after Parturition.—(1) The secretion of milk does not commence until about twenty-four hours after the birth of the young.

(2) Such secretion results from a purely secretory activity of the cells of the glandular epithelium, and is not connected with necrobiosis of either cytoplasm or nucleus.

Changes when Ovulation is not succeeded by

Pregnancy.—(1) A large growth of the mammary gland-tissue occurs, due to the mitotic divisions of its epithelial cells, and it results in the formation of true alveoli with single-layered walls. A substance which resembles milk is secreted in small quantities in these alveoli.

(2) The sebaceous and sweat-glands of the pouch area hypertrophy and become active.

(3) The above changes are very similar to, indeed indistinguishable from, those taking place during pregnancy.

Stimulus causing the growth of the Mammary Glands.—(1) The stimulus causing the hypertrophy of the mammary gland at any time is not a nervous one, but of the nature of an internal secretion carried by the blood.

(2) The seat of origin of the stimulus producing this growth at puberty, during heat, in virgins that have given milk, and in Dasyurus after ovulation not succeeded by pregnancy is neither in the fœtus nor in the placenta.

(3) The presence of a functional ovary is necessary for the growth of the gland at all times.

(4) The Corpus luteum is a ductless gland producing an internal secretion which is responsible for the attachment of the embryo to the wall of the uterus.

(5) (A) In Dasyurus, in the non-pregnant as well as in the pregnant animals, the formation and growth of the corpora lutea are intimately connected with the growth of the mammary gland. In the non-pregnant animals the secretion of the corpora lutea appears to be the only assignable cause of the growth of the gland.

(B) In the rabbit, (a) the presence of an ovary (with Corpora lutea) is necessary for the growth of the mammary gland; (b) the growth of the mammary gland follows on the experimentally induced production of Corpora lutea.

The general conclusion supported by the above evidence is that the Corpus luteum is a ductless gland producing a secretion which is the inciting cause of the growth of the mammary gland.

Cause of Milk Secretion.—There is at present no satisfactory theory of the causation of milk secretion. That of the removal by parturition of an inhibitory stimulus originating in the placenta or fœtus does not meet all cases.

Addendum.

Since the manuscript of this paper was sent for publication in January several communications dealing with the function of the corpus luteum and with the growth of the mammary gland have come under my notice, I propose therefore to refer briefly to some of these.

Tandler (**58**) and Foges (**54**) have come to the conclusion that "intra-uterine, pre-puberty, and puberty growth of the breasts is directly dependent on ovarian function" (vide **55**).

Frank and Unger (**55**) have reported that a series of growth changes occur in the virgin rabbit resulting in conditions "which were indistinguishable from those seen at the end of the first third of pregnancy." This fits in with the description I have given above of the changes that take place in the mammary apparatus of Dasyurus when ovulation is not succeeded by pregnancy. It is a further example of mammary growth that cannot be ascribed to the influence of either fœtus or placenta.

They further note that these virgin rabbits may have breasts more developed than those of the ninth or tenth day of pregnancy as described by Claypon and Starling (**31**). This further emphasises the criticism of the results obtained by these authors made in my paper. Precisely the same criticism, namely that in none of the experiments was any attention paid to, or allowance made for, this growth of the mammary glands in non-pregnant animals, applies also to the work of Foa (**53**) and Biedl and Koenigstein (**52**). These investigators performed a series of experiments somewhat like those of Claypon and Starling and arrived at more or less similar conclusions.

Basch (**51**) in 1909 put forward the view that an interrelation between fœtus and ovary brought about the hypertrophy of the mammary glands. The majority of these experiments were made on multiparous animals in which milk secretion had ceased, and in these cases placental extracts or implantation of placental tissue produced a flow of milk. Now multiparous animals for some considerable time after the cessation of milk secretion are useless for these growth experiments as the gland is already fully grown, and Biedl and Koenigstein report that abscesses in the breasts of such animals lead to milk being secreted. Basch does not clearly distinguish between milk secretion and hypertrophy of the gland, and milk secretion is no index of gland growth (vide Frank and Unger). All these experiments show is that the gland when it is fully formed may be stimulated to secretory activity by placental extract. This fact is of interest in itself and may have some bearing on the cause of milk secretion, but it has nothing to do with the formative growth of the gland, which, of course, had occurred before the experiments commenced. The author further describes one experiment on a virgin bitch in which two ovaries from a pregnant bitch were implanted subcutaneously into the back. In a fortnight there was an increase in the breasts, and in eight weeks (i.e. about the normal duration of pregnancy) they were equal in size to those of a late pregnant animal, although the implanted ovaries were degenerate. Placental extract was injected and was followed by the secretion of milk, and the bitch was able to suckle pups. In this case, again, no allowance was made for growth in non-pregnant animals, and further, Heape (**21**) has recorded cases of virgin bitches giving milk and suckling pups simply after copulation not followed by pregnancy. So that no definite inference can be drawn from this experiment.

Finally, it is only just to point out that L. Loeb (**56** and **57**), who has added a good deal of further valuable evidence in support of the theory that the corpus luteum plays an important part in the uterine changes of early pregnancy, found that he could not induce these changes by daily subcutaneous injection of corpus luteum extract from an homologous animal. Frank and Unger (loc. cit.) were also unsuccessful in their injections of rabbits' ovaries with fresh corpora lutea, but in spite of this came to the conclusion from other general considerations that "evidence points to the fact that the persistent corpus luteum of pregnancy may produce this breast growth."

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EXPLANATION OF PLATES 22 AND 23,

Illustrating Mr. Chas. H. O'Donoghue's paper on "The Growth-changes in the Mammary Apparatus of Dasyurus and the Relation of the Corpora Lutea thereto."

LIST OF COMMON REFERENCE LETTERS.

al. Alveolus. *c.* Coagulum (of colostrum-like substance). *c.m.* Cremaster muscle. *d.* Milk-duct. *d.o.* Opening of milk-duct on teat. *f.* Fibrillar cell. *fol.* Follicle of hair. *h.* Hair. *l.* Lymph-gland. *m.* Mitotic figure. *m.c.* Milk coagulum. *mu.* Muscles. *o.* Fat-globule. *seb.* Sebaceous gland. *sw.* Sweat-gland. *t.* Mammary tubule. *w.* Wall of alveolus.

PLATE 22.

Fig. 1.—Photo-micrograph (× about 90). Transverse section of the gland-area in a pouch young twenty weeks after birth (i.e. Series 2). One of the six strong hairs (*h.*) is projecting outward from its follicle

(*fol.*). Note the absence of hair from the other parts of the skin. The secondary or sebaceous outgrowth (*seb.*) and the tertiary or mammary outgrowth (*ter.*) are also shown, and the latter is cut again in its tubular part (*t.*)

Fig. 2.—Photo-micrograph (× about 16). Transverse section of teat and gland in the adult resting pouch (i.e. Series 3).

Fig. 3.—Photo-micrograph (× about 16). Transverse section of teat and gland just before attachment of embryos to uterus (i.e. Series 14). It shows the maximum development of the sebaceous glands (*seb.*) and at the base of the section a portion of the cremaster muscle (*c.m.*).

Fig. 4.—Photo-micrograph (× about 16).—Transverse section of teat and gland just before the birth of the young (i.e. Series 17). Comparison with fig. 3 shows that the sebaceous (*seb.*) and sweat-glands (*sw.*) are no further advanced, while the mammary glands have enlarged.

Fig. 5.—Photo-micrograph (× about 16). Transverse section of teat and gland with new-born young in the pouch (i.e. Series 18). The state of the mammary glands is practically the same as in fig. 4. It also shows a part of the lymph-gland (*l.*), which underlies the mammary gland and is more enlarged here than in the resting pouch.

Fig. 6.—Photo-micrograph (× about 16). Transverse section of teat and gland at the commencement of milk flow (i.e. Series 21). Owing to the way in which the teat was bent upon itself the section runs nearly transversely across it, and so shows clearly the six main ducts (*d.*) which open separately on its surface.

Fig. 7.—Photo-micrograph (× about 16). Transverse section of teat and gland when the young are thirty-four days old (i.e. Series 23). The ducts (*d.*) are dilated with milk (*m.c.*), as the animal was probably killed while feeding her young. The opening of two milk-ducts (*d. o.*) appear on the surface of the teat.

Fig. 8.—Photo-micrograph (× about 16). Transverse section of part of the mammary gland in full activity when the young are four months old (i.e. Series 25). This is magnified to the same extent as figs. 2–7, and shows the enormous hypertrophy undergone by the gland. The alveolar walls (*w.*) are reduced to mere lines.

Fig. 9.—Photo-micrograph (× about 16). Transverse section of teat and gland where ovulation has not been followed by pregnancy (Series 27.) Note the advanced state of the gland, it is more advanced than that in fig. 3.

Fig. 10.—Photo-micrograph (× about 16). Transverse section of teat and gland where ovulation has not been followed by pregnancy (Series 30). Comparison will show that the gland is in a more advanced state than any of those before parturition, and it corresponds most nearly to that in fig. 6.

PLATE 23.

[The drawings were all made at the same magnification by means of a camera lucida with a $\frac{1}{12}$-in. oil-immersion objective and a No. 2 Zeiss ocular.]

Fig. 11.—(× about 560.) Transverse section of the mammary outgrowth in the pouch young twenty weeks after birth (i. e. Series 2) showing the two-layered condition of its wall. The fibrillar cells (*f.*) of the conjunctive tissue also show and it will be seen there is no membrana propria.

Fig. 12.—(× about 560.) Transverse section of part of one of the main ducts in the teat while the gland is at rest (i. e. Series 3). The wall is composed of a stratified epithelium four or five cells thick, and a coagulum (*c.*) is present in the lumen.

Fig. 13.—(× about 560.) Transverse section of two tubules of the resting adult mammary gland (i. e. Series 3). The wall is characteristically two-layered; a coagulum (*c.*) is present in the lumen. The fibrillar cells (*f.*) do not form a membrana propria.

Fig. 14.—(× about 560.) Transverse section of a tubule of gland during growth, when ovulation was not followed by pregnancy (i. e. Series 28). Solid bud-like outgrowths are present in, one of which a mitotic figure (*m.*) is shown.

Fig. 15.—(× about 560.) Transverse section of two tubules at the stage of maximum growth activity (i. e. Series 9). Several stages of mitoses (*m*) can be recognised and the coagulum (*c*) is present.

Fig. 16.—(× about 560.) Transverse section of a fully formed alveolus some time after the attachment of the embryo to the uterus (i. e. Series 16). The wall is single-layered and no membrana propria is present.

Fig. 17.—(× about 560.) Transverse section of an alveolus at the commencement of milk secretion (i. e. Series 21). The cells are granular, and a fat-globule (*o.*) is present in one of them. The lumen contains milk (*m.c.*)

Fig. 18.—(× about 560.) Transverse section of a corner between three alveoli when the gland is fully active and its lumen distended with milk (i. e. Series 25). This shows the enormous increase in the size of the alveoli. Enclosed between the single-layered alveolar walls (*w.*) are several fibrillar cells (*f.*), which are now approximated to the alveoli. An enormous quantity of milk coagulum (*m. c.*) is present. The cells are granular and contain fat-globules (*o.*).